

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A recombinant thermostable DNA polymerase which is characterized in that

a) said thermostable DNA polymerase comprises:

i) the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is not Glu, and

ii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; or

~~b) — said thermostable DNA polymerase comprises:~~

~~i) — the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Arg and "Xaa" at position 7 is Val or Ile; and~~

~~ii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Arg.~~

2. (Previously presented) The recombinant thermostable DNA polymerase of claim 1 wherein said DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and

10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is not Glu; and

said nucleotides are dideoxynucleotides and said level of discrimination is at least 3-fold lower than that of said polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu.

3. (Original) The recombinant thermostable DNA polymerase of claim 2 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.

4. (Canceled)

5. (Canceled)

6. (Previously presented) The recombinant thermostable DNA polymerase of claim 1 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid except Glu, and "Xaa" at position 6 is Ser or Ala.

7. (Previously presented) The recombinant thermostable DNA polymerase of claim 1 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

8. (Previously presented) The recombinant thermostable DNA polymerase of claim 7 wherein said "Xaa" at position 4 of the thermostable DNA polymerase is Lys.

9. (Canceled)

10. (Canceled)

11. (Currently amended) A nucleic acid sequence encoding a recombinant thermostable DNA polymerase which is characterized in that

a) said thermostable DNA polymerase comprises:

i) the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is not Glu, and

ii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; or

~~b) — said thermostable DNA polymerase comprises:~~

~~—— i) — the amino acid sequence the amino acid sequence~~

~~LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Arg and "Xaa" at position 7 is Val or Ile; and~~

~~—— ii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Arg.~~

12. (Currently amended) The nucleic acid sequence of claim 11 wherein said DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is not Glu; and

said nucleotides are dideoxynucleotides and said level of discrimination is at least 3-fold lower than that of said polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu.

13. (Original) The nucleic acid sequence of claim 12 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.

14. (Canceled)

15. (Canceled)

16. (Previously presented) The nucleic acid sequence of claim 12 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid except Glu, and "Xaa" at position 6 is Ser or Ala.

17. (Previously presented) The nucleic acid sequence of claim 12 which is characterized in that

a) said thermostable DNA polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

18. (Previously presented) The nucleic acid sequence of claim 17 wherein the "Xaa" at position 4 of the thermostable DNA polymerase is Lys.

19. (Canceled)

20. (Canceled)

21. (Currently amended) A method of DNA sequencing which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 4 is not Glu, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; ~~or~~

~~ii) said thermostable DNA polymerase comprises:~~

~~1) the amino acid sequence~~

~~LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Arg and "Xaa" at position 7 is Val or Ile; and~~

~~2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Arg;~~

b) providing a dye-terminator labeled with a negatively charged fluorescent dye;

and

c) performing a dye-terminator sequencing reaction.

22. (Currently amended) The method of claim 21 wherein said nucleotides are dideoxynucleotides and said level of discrimination is measured by determining the ratio of the concentration of a dideoxynucleotide labeled with a fluorescein dye required for 50% inhibition of DNA synthesis versus the concentration of an unlabeled dideoxynucleotide required for 50% inhibition.

23. (Original) The method of claim 22 wherein said ratio is 4 or less.

24. (Canceled)

25. (Canceled)

26. (Previously presented) The method of claim 21 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

27. (Previously presented) The method of claim 26 wherein the "Xaa" at position 4 of the thermostable DNA polymerase is Lys.

28. (Canceled)

29. (Canceled)

30. (Canceled)

31. (Currently amended) A method of producing labeled DNA which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; or

~~ii) said thermostable DNA polymerase comprises:~~

_____ 1) _____ the amino acid sequence
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any
amino acid except Arg and "Xaa" at position 7 is Val or Ile; and

_____ 2) said thermostable DNA polymerase has a level of discrimination
against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in
comparison to a polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Arg;

- b) providing a nucleotide labeled with a fluorescein family dye; and
- c) performing a DNA synthesis reaction.

32. (Currently amended) A method of producing labeled primer extension
products which comprises:

- a) providing a thermostable DNA polymerase characterized in that
 - i) said thermostable DNA polymerase comprises:

1) the amino acid sequence
LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and
10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at
position 7 of said sequence is Ile and "Xaa" at position 4 is any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination
against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in
comparison to a polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Glu;

3) said polymerase also comprises the second amino acid sequence
SerGlnIleXaaLeuArg(Val/Ile) (SEQ ID NO: 18) where "X" is any amino acid except Glu, and

4) said polymerase has a level of discrimination against
incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in
comparison to the polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Glu; or

- _____ ii) _____ said thermostable DNA polymerase comprises:

_____ 1) _____ the amino acid sequence
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any
amino acid except Arg and "Xaa" at position 7 is Val or Ile; and

_____ 2) said thermostable DNA polymerase has a level of discrimination
against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in
comparison to a polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Arg;

_____ 3) said polymerase also comprises the second amino acid sequence
SerGlnIleXaaLeuArg(Val/Ile) (SEQ ID NO: 18) where "X" is any amino acid except Glu, and

_____ 4) said polymerase has a level of discrimination against
incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in
comparison to the polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Arg; and

- b) providing a ribonucleotide labeled with a fluorescein family dye; and
- c) performing a primer extension reaction.

33. (Currently amended) A kit for DNA sequencing which comprises:

- a) a thermostable DNA polymerase characterized in that
 - i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and
10 of said sequence are any amino acid residue, "Xaa" as position 4 can be any amino acid
except Glu, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile, and

2) said thermostable DNA polymerase has a level of discrimination
against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in
comparison to a polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Glu; or

- _____ ii) said thermostable DNA polymerase comprises:

_____ 1) the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Arg and "Xaa" at position 7 is Val or Ile; and

_____ 2) _____ said thermostable DNA polymerase has a level of

discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Arg; and

b) a terminator labeled with negatively-charged fluorescent dye.

34. (Original) The kit of claim 33 wherein said reduced level of discrimination is measured by determining the ratio of the concentration of ddNTP labeled with a fluorescein family dye required for 50% inhibition of DNA synthesis compared to that for an unlabeled ddNTP and said ratio is 4 or less.

35. (Original) The kit of claim 34 wherein said amino acid sequence comprises: LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

36. (Previously presented) The kit of claim 35 wherein the "Xaa" at position 4 of the thermostable DNA polymerase is Lys.

37. (Canceled)

38. (Canceled)

39. (Currently amended) A kit for a DNA extension reaction, the kit comprising:

a) a thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and

10 of said sequence are any amino acid residue, "Xaa" as position 4 can be any amino acid except Glu, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; or

~~ii) said thermostable DNA polymerase comprises:~~

~~1) the amino acid sequence~~

~~LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Arg and "Xaa" at position 7 is Val or Ile; and~~

~~2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Arg.~~

40. (Currently amended) The kit of claim 39 wherein said thermostable DNA polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" as position 4 can be any amino acid except Glu, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and said level of discrimination is at least 5-fold lower than that of a said thermostable DNA polymerase whose sequence is identical to said thermostable DNA polymerase except that position 4 is Glu.

41. (Previously presented) The kit of claim 40 wherein said recombinant thermostable DNA polymerase is characterized in that the thermostable DNA polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

42. (Previously presented) The kit of claim 41 wherein the "Xaa" at position 4 of the thermostable DNA polymerase is Lys.

43. (Canceled)

44. (Canceled)

45. (Currently amended) A kit for producing labeled DNA which comprises:

a) a thermostable DNA polymerase characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu; or

~~ii) said thermostable DNA polymerase comprises:~~

~~1) the amino acid sequence~~

~~LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Arg and "Xaa" at position 7 is Val or Ile; and~~

~~2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Arg; and~~

b) a nucleotide labeled with a negatively-charged fluorescent dye.

46. (Previously presented) The kit of claim 45 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO: 3), whereby "Xaa" at position 4 is any amino acid except Glu.

47. (Previously presented) The kit of claim 45 wherein the "Xaa" at position 4 of the thermostable DNA polymerase is Lys.

48. (Canceled)

49. (Canceled)

50. (Currently amended) A kit for producing labeled primer extension products which comprises:

a) a thermostable DNA polymerase which is characterized in that

i) said thermostable DNA polymerase comprises:

1) the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 9, and 10 of said sequence are any amino acid residue, "Xaa" at position 6 is Ala or Ser and "Xaa" at position 7 of said sequence is Ile and "Xaa" at position 4 is any amino acid except Glu, and

2) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to a polymerase whose sequence is identical to that of said thermostable DNA polymerase except that "Xaa" at position 4 is Glu;

3) the thermostable DNA polymerase also comprises the second amino acid sequence SerGlnIleXaaLeuArg(Val/Ile) (SEQ ID No: 18) where "Xaa" is any amino acid except Glu;

4) the thermostable DNA polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the thermostable DNA polymerase; or

~~ii) said thermostable DNA polymerase comprises:~~

- _____ 1) the amino acid sequence
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any
amino acid except Arg and "Xaa" at position 7 is Val or Ile; and
- _____ 2) said thermostable DNA polymerase has a level of discrimination
against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in
comparison to a polymerase whose sequence is identical to that of said thermostable DNA
polymerase except that "Xaa" at position 4 is Arg;
- _____ 3) the thermostable DNA polymerase also comprises the second
amino acid sequence SerGlnIleXaaLeuArg(Val/Ile) (SEQ ID No: 18) where "Xaa" is any amino
acid except Glu;
- _____ 4) the thermostable DNA polymerase has a level of discrimination
against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in
comparison to the thermostable DNA polymerase; and
- b) a ribonucleotide labeled with a fluorescein family dye.

51. (Previously presented) The kit of claim 50 wherein said amino acid
sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at
position 4 is any amino acid except Glu.

52. (Previously presented) The kit of claim 51 wherein the "Xaa" at position 4
of the thermostable DNA polymerase is Lys.

53. (Canceled)

54. (Canceled)